SUPPLEMENTARY FIGURES

Supplementary Figure 1: Up-regulation of FoxP3 in expanded Treg. (A) gating for flow sorting of Treg and Teff cells. (B) Fresh PBMC and expanded cells (round 2) were stained for CD3, CD4, CD25, CD127, and FoxP3. Treg were gated on $CD4^{+}CD25^{hi}CD127^{-}$; Teff were gated on $CD4^{+}CD25^{-}$. FoxP3 MFI (upper right corner of each panel) in expanded Treg is compared with that in fresh bulk T cells, fresh Treg, fresh Teff, and expanded Teff. Expanded Treg show significantly increased FoxP3 expression.

Supplementary Figure 2: Expanded Treg and Teff significantly down-regulate CD52 expression. T cells from LN were stained for CD3, CD4, CD25, CD127 and CD52. CD52 MFI (shown in the upper right corner of each panel) was analyzed for bulk T cells, fresh (unexpanded) Treg (gated for $CD4^{+}CD25^{hi}CD127^{-}$) and fresh (unexpanded) Teff (gated for $CD4^{+}CD25^{-}$). In the same experiment, Treg and Teff were analyzed for CD52 after round 2 of expansion. Expanded cells showed a 10-fold decrease in CD52 expression after the second round of expansion.

Supplementary Figure 3: Alemtuzumab does not kill expanded Treg

T cells were incubated with alemtuzumab at the concentrations shown to determine apoptosis and killing of cells. Fresh T cells (top row) showed increasing 7-AAD and Annexin-V staining as the concentration of alemtuzumab increased. In contrast, expanded Treg (middle row) showed a relatively low baseline level of Annexin-V and 7-AAD staining, which remained unchanged, irrespective of the concentration of alemtuzumab. Expanded Teff (bottom row) had a higher baseline level of Annexin-V and 7-AAD staining, but did not show any increase when alemtuzumab was added. Data are representative of 2 separate experiments.

Supplementary Figure 4: Alemtuzumab-containing serum, taken immediately after mAb infusion, does not kill expanded Treg.

Monkey serum was drawn pre-alemtuzumab, immediately after a second dose (d5), immediately before and after a third dose (d12), and 1 week after the third dose (d20). These sera were incubated with freshly-isolated monkey T cells and expanded autologous Treg. Histograms of Annexin-V and 7-AAD staining are shown. Fresh cells showed an increase in apoptosis and cell killing in response to high concentrations of alemtuzumab in the blood (d5 post-alemtuzumab, d12 post-alemtuzumab), as well as when exposed to pre-alemtuzumab serum to which 10 μg/ml of alemtuzumab had been added (far right). Values returned to baseline levels of apoptosis and killing when fresh T cells were incubated with serum obtained 1 week after alemtuzumab infusion (d12pre, d20), indicating that serum contained high concentrations of alemtuzumab early after infusion. Expanded autologous Treg showed no increase in apoptosis or killing when exposed to a high concentration of alemtuzumab in serum, whether the serum was drawn from an alemtuzumab-treated monkey, or whether alemtuzumab had been added to the serum *in vitro* (far right).